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ULTRASTRUCTURAL RELATIONSHIP OF MELANOCYTES TO MAST CELLS AND "MELANOPHAGES" IN A LESION OF ALOPECIA MUCINOSA*

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ABSTRACT

Mast cells were observed in the basal layer of epidermis and external hair sheaths in a case of alopecia mucinosa with ultrastructural examination, paralleling the light microscopic observation of cells containing metachromatic granules in these areas. Intermediate cells which combined features of mast cells and melanocytes were also present in focal areas of the basal layer. These were characterized ultrastructurally by the presence of both mast cell granules and melanosomes in their cytoplasm and by the presence of atypical granules combining ultrastructural features of both granule types.

An electron density consistent with melanization was noted in some mast cell granules.

Cells were present in the dermis containing individual melanosomes in various stages of melanization, melanosome complexes, or both individual melanosomes and melanosome complexes. True vacuoles (phagosomes) containing melanin or endocytotic vesicles containing melanin were not observed. Melanosomes in the intercellular spaces were extremely rare.

These observations support the hypotheses a) that mast cells and melanocytes may be histogenetically related, and b) that at least some "melanophages" are melanocytes in which melanosome complexes form by intracellular reorganization.

Melanocytes are considered to be morphologically and histogenetically distinct from connective tissue cells. Although "melanophages" characteristically contain melanin and mast cells occasionally contain melanin (1, 2, 3), both of these cell types are considered to be of mesodermal rather than neural crest origin and the melanin they contain is usually assumed to be phagocytized rather than synthesized (4, 5, 6).

Previous studies from our laboratory (3, 7, 8, 9) presented morphologic, enzymatic and histochemical evidence for a possible histogenetic relationship between mast cells, melanocytes and "melanophages" and cited embryologic studies supporting this hypothesis. In one of these studies (7) dendritic cells with metachromatic granules were observed in the epidermis and external hair sheaths in lesions of alopecia mucinosa.

The present report concerns ultrastructural observations of a case of alopecia mucinosa related to our previous studies and to the question of histogenetic relationships of melanocytes.

MATERIALS AND METHODS

Tissue for light and electron microscopy was obtained from a 1.5 cm infiltrated area of alopecia, which had been present for 18 months, on the temporal scalp of a 58-year-old man. The patient was otherwise in good health and had no clinical or laboratory evidence of lymphoma. He was a brunet of Portuguese descent with no clinical pigmentary abnormalities. There was no nevus or freckling in the area biopsied.

Paraffin sections from tissue fixed in 10% formalin were stained with H and E and Giemsa stains.

The specimen for electron microscopy was prefixed in Karnovsky's fixative, postosmicated and then dehydrated and embedded in araldite. Thin sections were stained with lead citrate and uranyl acetate and were examined with an RCA EMU-3 electron microscope.

RESULTS

Light microscopy. The epidermis and external hair sheaths showed slight acanthosis and focal areas of spongiosis; the external hair sheaths and sebaceous glands showed foci of mucinous degeneration. A moderately dense infiltrate was present in the dermis composed of histiocytes, lymphocytes, plasma cells, mast cells and "melanophages."

Scattered cells with peripheral clear zone were present in the basal layer of epidermis and external hair sheaths, which contained metachromatic granules; these were similar to cells illustrated in our previous study (7). They were present in areas of epidermis and external hair sheaths which either appeared to be otherwise normal, or which showed slight spongiosis. There was no inflammatory exocytosis.

The histologic diagnosis was alopecia mucinosa.

Electron microscopy. In focal areas of the basal layer of epidermis and external hair sheaths, oval (Fig. 1a) or elongated cells were present which had the ultrastructure of mast cells. They had characteristic mast cell granules with maximum

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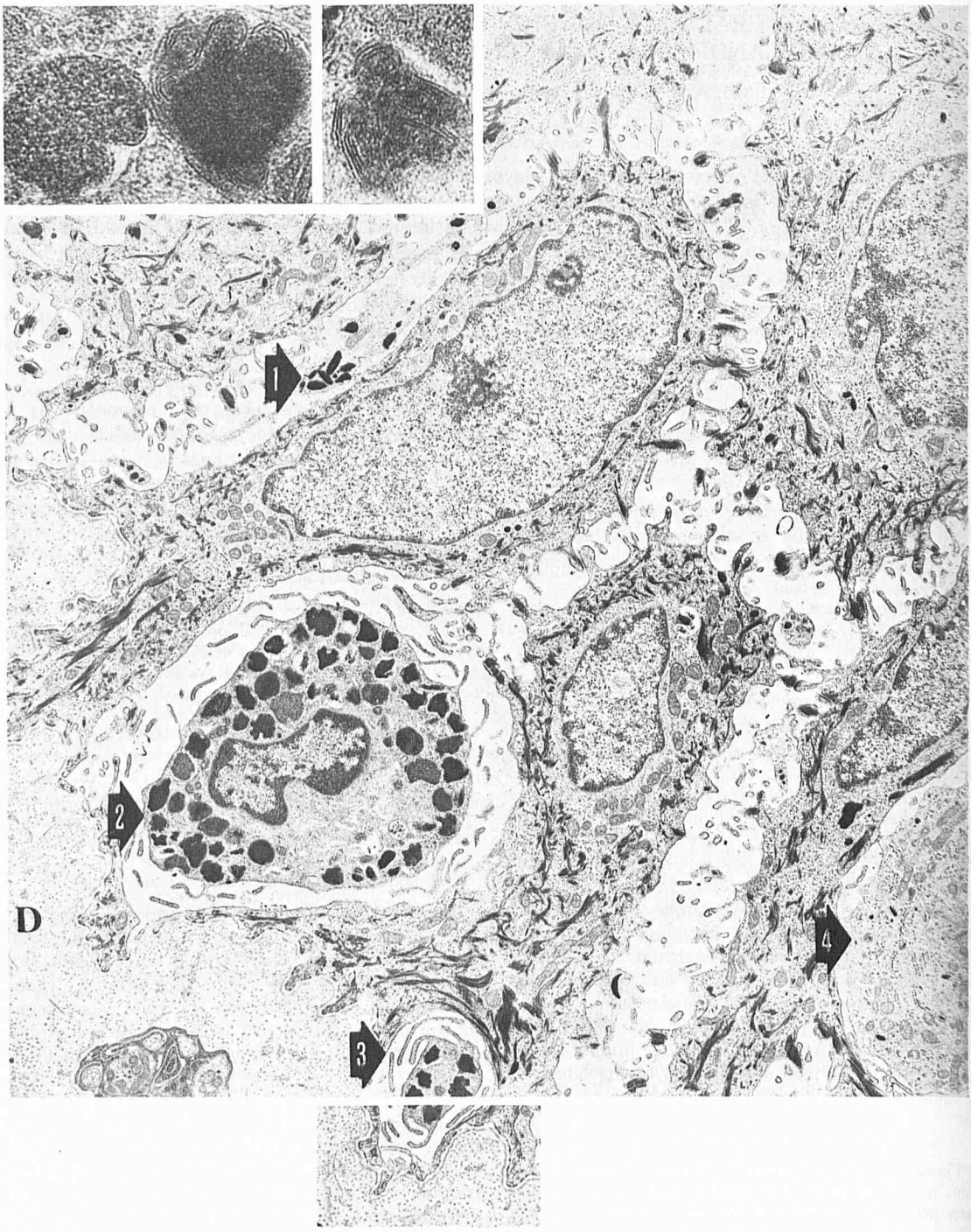

FIG. 1a. Field shows basal mast cells in the epidermis (arrows 2 and 3) and portions of nearby basal melanocytes (arrows 1 and 4). Mast cells have typical microvilli and contain granules 200 nm to 800 nm in diameter having the characteristic ultrastructure of human mast cell granules: granular material, parallel lamellae and lamellar scrolls, as illustrated (inset) at higher magnification. One basal melanocyte (arrow 1) contains melanosomes which are larger than normal. Slight spongiosis but no inflammatory exocytosis is present. (D = dermis.) × 7100; inset: × 50,000.

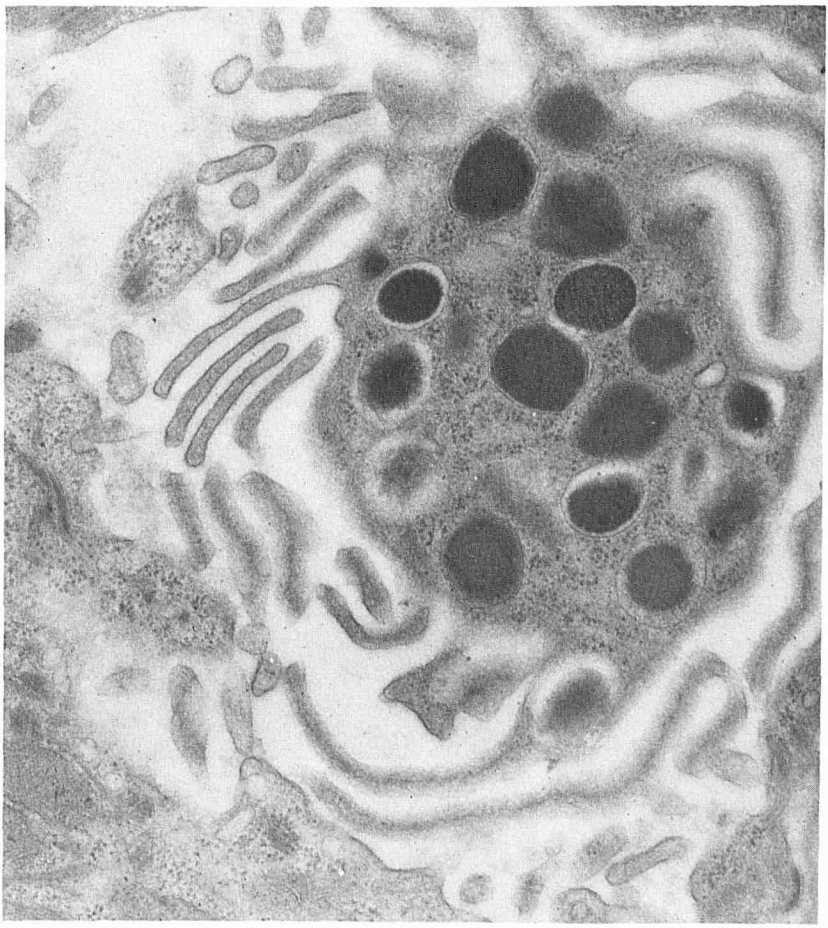

FIG. 1b. Basal mast cell is illustrated having greatly elongated microvilli. × 27,000.

diameter ranging from 200 nm to 800 nm; these had an ultrastructure composed of granular material, parallel lamellae and lamellar scrolls (Fig. 1a, inset), with an average distance of 90 Å between lamellae. The basal mast cells also showed typical microvilli, some of which were long enough to be considered dendrites (Fig. 1b). Microfilaments in varying degrees of profusion were present in the cytoplasm of the basal mast cells.

The basal layer of the epidermis and external hair sheaths also showed many normal melanocytes containing melanosomes in various stages of melanization (Fig. 2).

In addition to typical mast cells and typical melanocytes in the basal layer, cells which combined ultrastructural features of mast cells and melanocytes were present in focal areas of the basal layer. Some of these intermediate cells most closely resembled normal melanocytes and others most closely resembled normal mast cells, with gradations in between. They contained varying numbers of melanosomes and mast cell granules as well as atypical granules combining ultrastructural features of mast cell granules and melanosomes (Figs. 3 and 4). The maximum diameter of these atypical granules ranged from 200 nm to 300 nm, the size of small mast cell granules. Some appeared to be highly melanized and their size and shape conformed to that of small mast cell granules (Fig. 4a). The less electron dense atypical granules showed varying combinations of cross-linked fibers, parallel and non-parallel lamellae and lamellar scrolls with varying degrees of electron density (Figs. 4b to 4j). The average distance between parallel lamellae approximated that observed in normal mast cells granules: 90 Å. Occasional granules were present in intermediate cells which had the structure (Fig. 4c) of mast cell granules following histamine release (10), but with small areas showing the fiber arrangement of melanosomes.

Some intermediate cells in the basal layer had ultrastructural features approaching those of normal mast cells (Fig. 5). The granules of these cells were fairly uniform with maximum diameter from 200 nm to 300 nm; most of them did not have a clear outer membrane; they were composed of particulate material, parallel lamellae and lamellar scrolls. Features of these granules were similar to those which Kobayasi *et al.* (10)

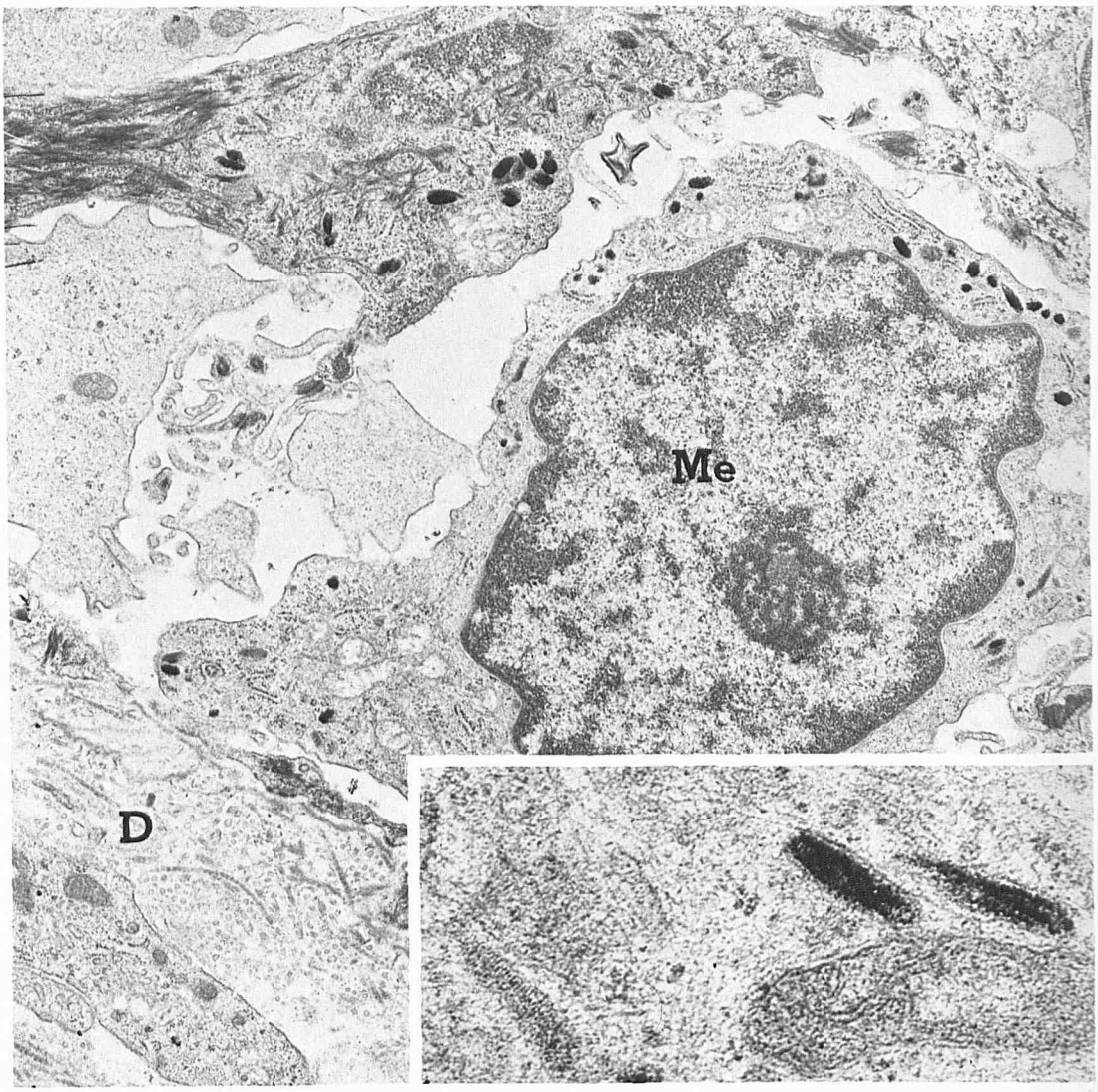

FIG. 2. A normal epidermal melanocyte is illustrated (Me); higher magnification (inset) demonstrates melanosomes characteristic of normal epidermal melanocytes with cross-linked fibers in various stages of melanization. (D = dermis.) × 19,500; inset: × 66,000.

described as characterizing immature mast cell granules (Fig. 5a, III; Fig. 5b, IV). However, melanosomes (Fig. 5a, II) and atypical granules (Fig. 5a, II) were also present in intermediate cells of this type. They also contained mast cell granules with foci of electron density consistent with melanin (Fig. 5b, II) and basal dense plates (fig. 5b, III) similar to those described in basal melanocytes by Tarnowski (11).

Indeterminate dendritic cells (Fig. 6) not containing specialized cytoplasmic organelles, as well as normal Langerhans cells, were present in the epidermis.

Electron microscopic examination of the dermal infiltrate confirmed the identity of the cells observed by light microscopy. Many cells were present which contained melanosome complexes; cells were also present showing both individual melanosomes in various stages of melanization and melanosome complexes (Fig. 7a), or varying numbers of individual melanosomes (Fig. 7b, c, d). Some cells contained organelles which could represent stages of transformation of an individual melanosome into a melanosome complex (Fig. 7a, insets). Melanosomes or frements of melanocytes were not observed in endocytotic vesicles or in phagosomes (i.e., in true vacuoles). Melanosomes in the intercellular spaces were extremely rare.

DISCUSSION

Ultrastructural relationship of mast cells to melanocytes. The observations of this report add to the evidence that there is a histogenetic rela-

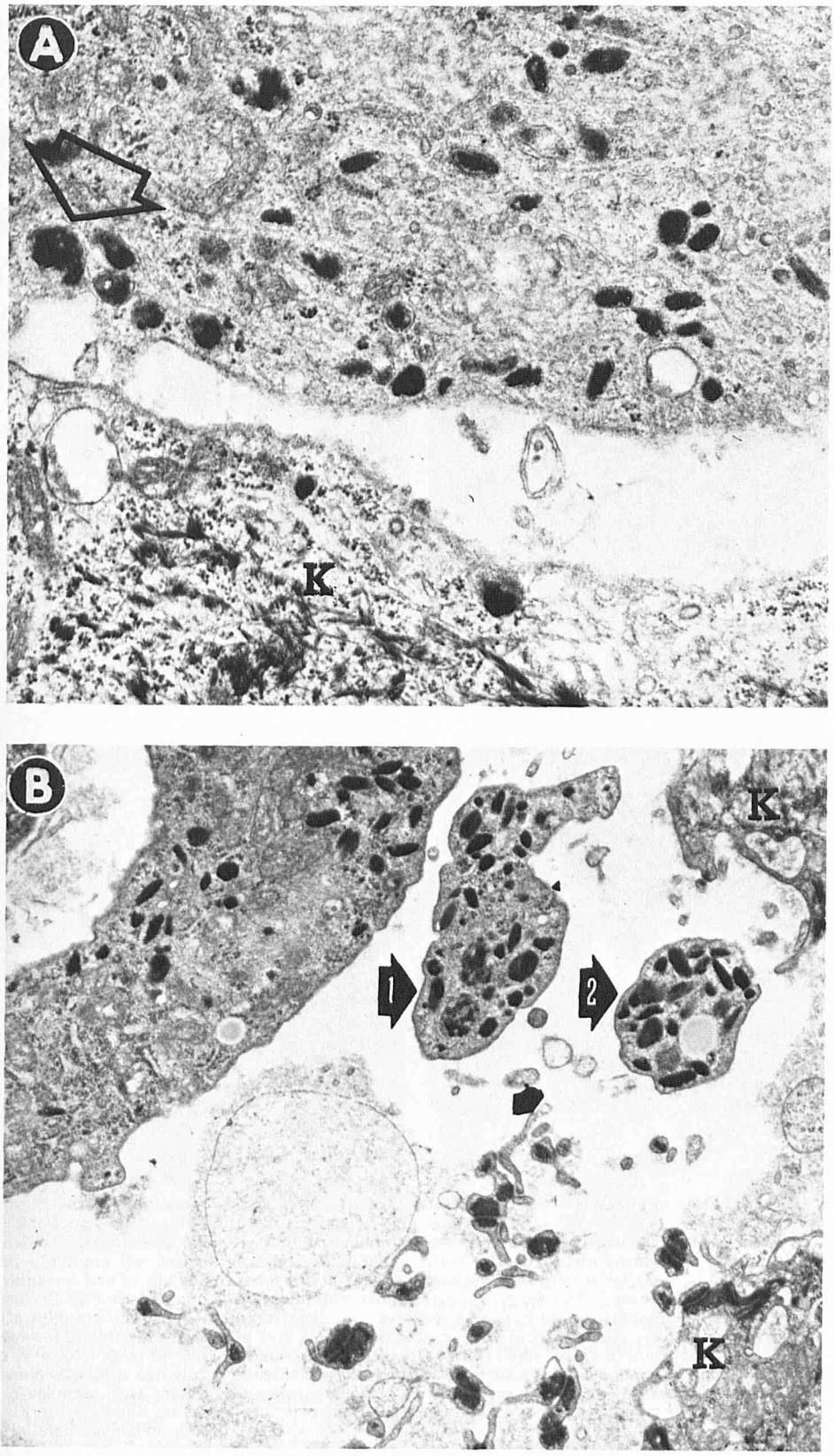

FIG. 3. Fields illustrated in Figs. 3a and 3b show areas of intermediate cells resembling melanocytes more than mast cells. Most of the granules present are normal melanosomes. In areas indicated by arrows atypical granules are present combining features of melanosomes and mast cell granules. (K = keratinocyte) High magnification detail of fields containing these atypical granules is presented in Figs. 4b, c, and h. a: × 22,000; b: × 13,000.

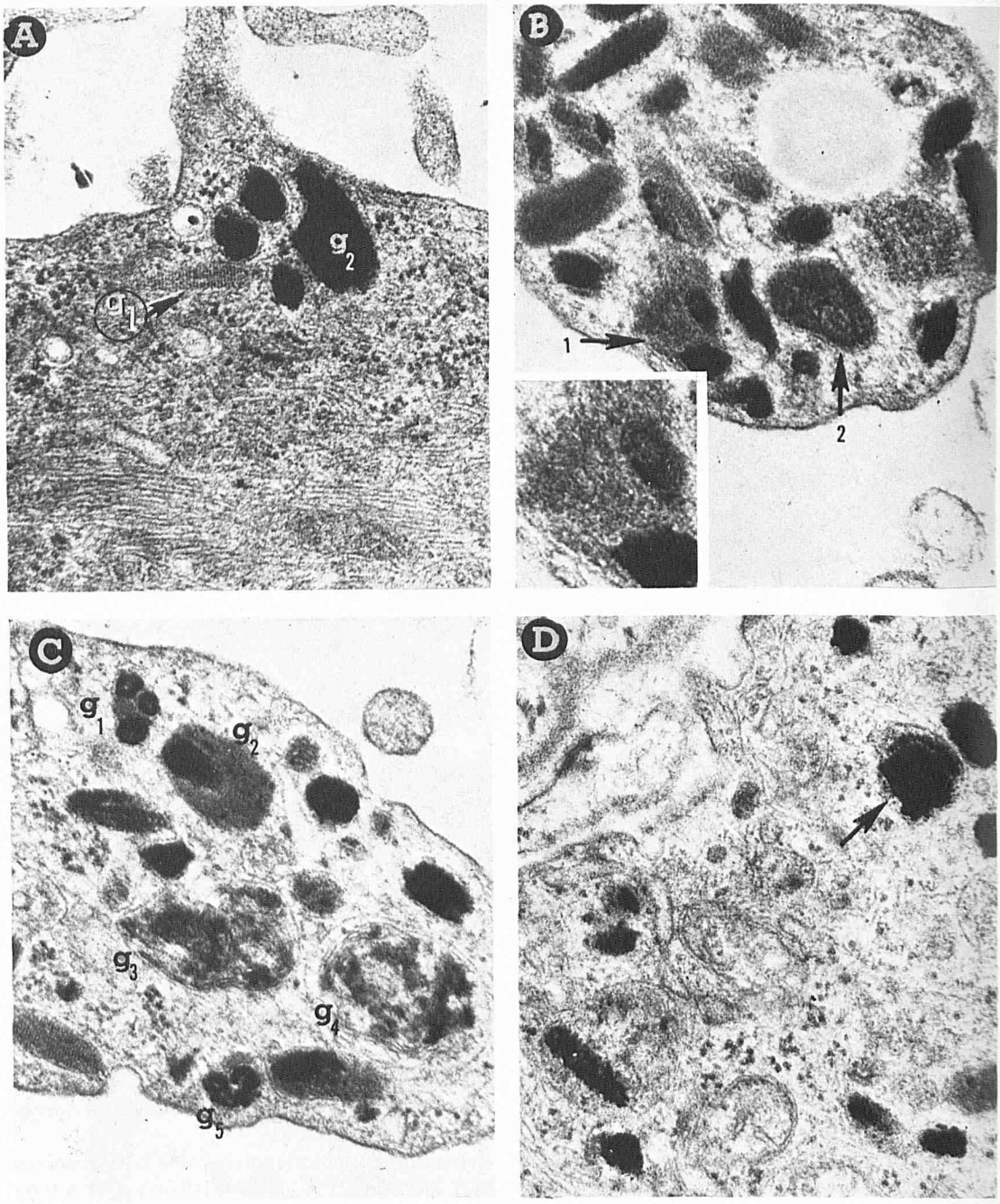

FIG. 4. High magnification illustrates ultrastructural detail of atypical granules in transitional cells.

FIG. 4a shows electron-dense organelle (g_2) having size and shape of mast cell granule. Adjacent to it is a normal non-melanized melanosome (g_1). In Figs. 4b, c there are normal or slightly atypical melanosomes as well as distinctly atypical granules combining ultrastructural features of melanosomes and mast cell granules—particulate material, parallel lamellae, lamellar scrolls with apparent melanization, cross-linked fibers and irregular areas of melanization (Fig. 4b, arrows 1 and 2; Fig. 4c, g_1, g_2, g_5). Higher magnification of organelle in Fig. 4b (inset) shows melanizing lamellar scroll. Granules g_3 and g_4 in Fig. 4c have the ultrastructure of mast cell granules after histamine release. They are relatively electron-lucid with scattered lamellae and particulate material. However, in one area of granule g_3 there is a group of cross-linked fibers. Organelles illustrated in Figs. 4d to 4i, inclusive, also combine ultrastructural features of melanosomes and mast cell granules. In Fig. 4d (arrow) an organelle shows a large area of electron-density, a dense lamella and cross-linked fibers. Organelles are present with lamellae of variable electron density suggesting that they are undergoing progressive melanization. These are illustrated in Figs. 4e, g_1; 4f, g, arrows; 4h, g_1; 4j, arrow. Higher magnification (Fig. 4j, inset) shows detail of non-melanized parallel lamellae. Elsewhere in Fig. 4j there is a relatively normal melanosome (m). Fig. 4i illustrates a normal melanosome next to an atypical organelle (arrow) with a complex pattern of lamellae, particulate material and melanin deposition. Figs. 4a to 4j, inclusive: × 73,000. Fig. 4c, inset: × 167,000. Fig. 4j, inset: × 142,000.

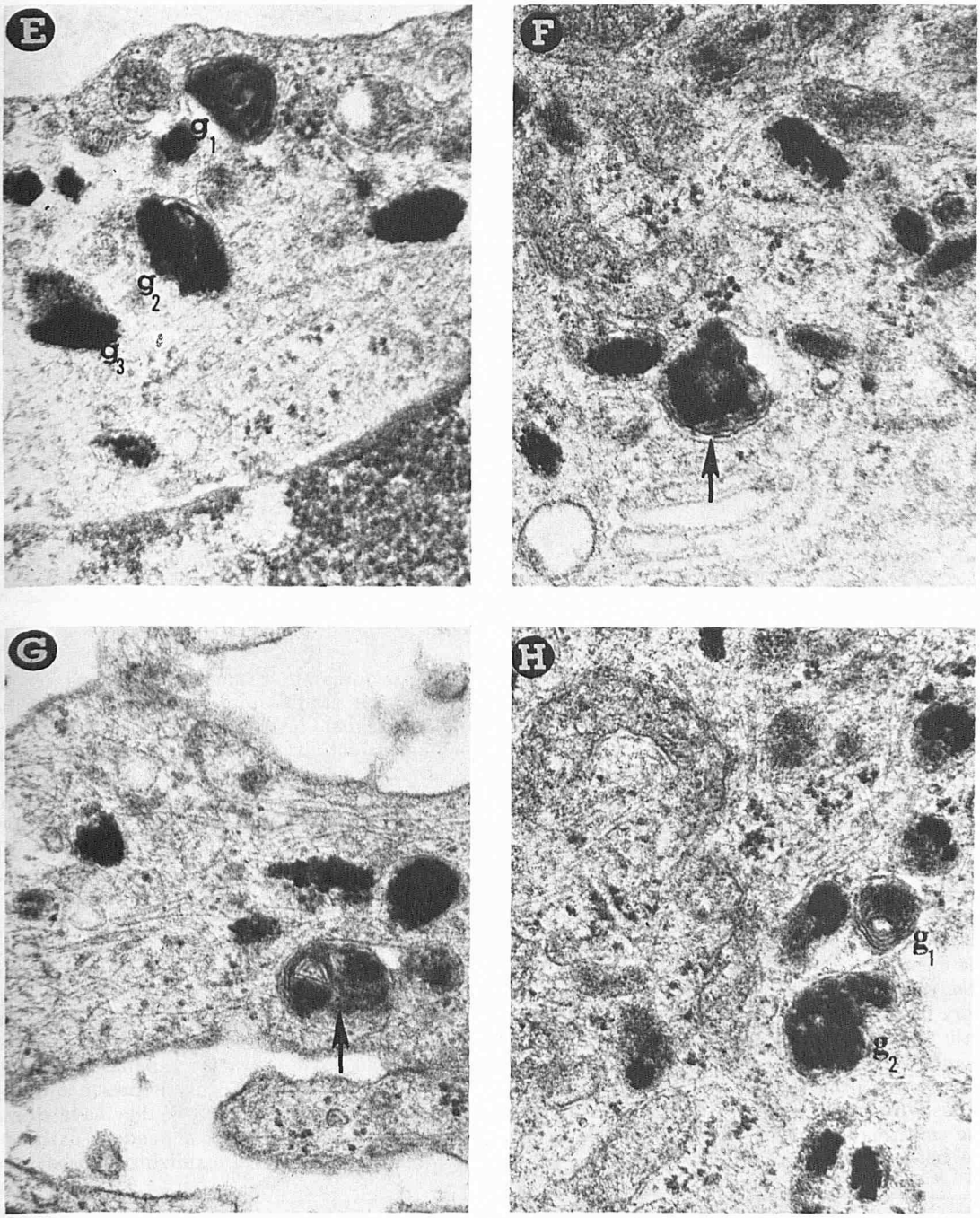

FIG. 4e–h.

tionship between mast cells and melanocytes. Mast cells have been described in the epidermis of normal skin (12) but they are not generally present as a component of inflammatory exocytosis. In the lesions of alopecia mucinosa described in this report and in our previous report (7), mast cells were observed in the epidermis and external hair sheaths showing no inflammatory exocytosis. The following facts are against the possibility that our observations were based only on a phagocytic interchange between mast cells and melanocytes (heterophagocytosis): first, the presence of granules with the infrastructure of mast cell granules with foci of electron density consistent with melanization (Fig. 5b, II), and second, the presence of granules combining ultrastructural features of both mast cell granules and melanosomes (Figs. 3 and 4). The lamellar pat-

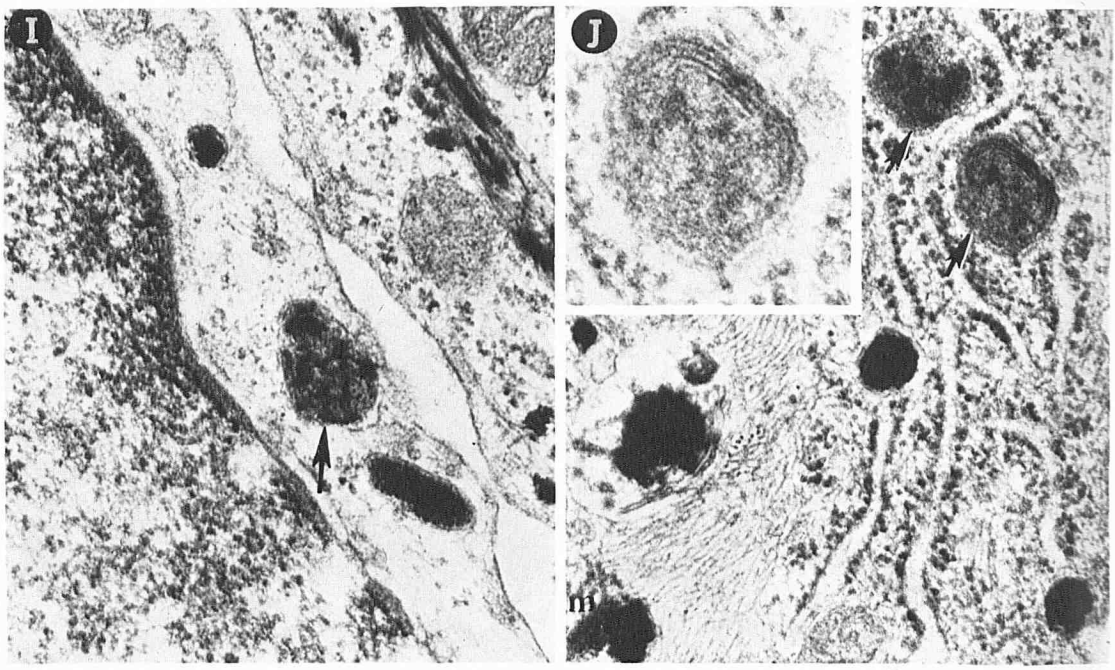

FIG. 4i and j.

tern in these granules was different from the lamellar pattern in normal melanosomes seen in longitudinal or cross section, and resembled the lamellar pattern of mast cell granules. However, the cross-linkage of the fibers resembled that of melanosomes. The patient was a brunet with no pigmentary abnormalities, excluding a generalized genetic pigmentary abnormality.

In earlier studies we cited light microscopic and ultrastructural parallels between mast cells of human mastocytosis and dermal nevus cells (3), as well as light microscopic parallels between canine mastocytoma and canine melanoma (8).

Enzymatic considerations. Studies in our laboratory have shown that mast cells have the enzymatic potential to oxidize tyrosine or dopa to melanin (3, 13) and that this potential is based on the presence of peroxidase (14–18). Biochemical studies with purified mammalian peroxidase (19) have confirmed the ability of this enzyme to oxidize both tyrosine (in the presence of dopa or dihydroxyfumarate co-factor) and dopa to melanin. Other studies in our laboratory (20) have indicated that histochemical demonstration of peroxidase activity in mast cells exhibits latency; some damage must be produced to effect adequate substrate–enzyme interaction. Our demonstration of peroxidase activity in mast cells is supported by studies of Wachstein and Maisel (21), Montagna and Noback (22) and other investigators.

Histochemical (14–18, 23) and biochemical (24) studies in our laboratory have demonstrated peroxidase activity in melanocytes and have indicated that peroxidase activity in some melanocytes exhibits latency; however, our studies have been unable to demonstrate the existence of a mammalian aerobic oxidase capable of oxidizing tyrosine to melanin (even in the presence of dopa co-factor) (15–18, 24), although they have demonstrated an aerobic, copper-dependent oxidase in melanocytes capable of oxidizing dopa to mel-

FIG. 5. The two cells illustrated represent intermediate cells in the basal layer resembling mast cells more than melanocytes.

The majority of the granules in these cells are relatively uniform in size and shape and resemble small immature mast cell granules as described by Kobayasi *et al.* (10). Low magnification (Figs. 5a, I and 5b, I) provides overall views of these cells. Figs. 5a, II and III show detail of granules indicated by arrows in Fig. 5a, I. In Fig. 5a, II one granule (g_1) shows structural features similar to granule illustrated in Fig. 4g (arrow), and other granule (g_2) has appearance of fully melanized melanosome. (mi = mitochrondrion.) Granule illustrated in 5a, III shows lamellar structure and fine granular material as well as absence of distinct limiting membrane. In Fig. 5b, I granule indicated by arrow is presented in higher magnification in Fig. 5b, II and shows multiple foci of apparent melanization and a lamellar scroll characteristic of mast cell granules at one pole; adjacent non-melanized mast cell granule also shows polar lamellar scrolls. High magnification of area shown in 5b, III illustrates basal dense plates (arrows) similar to those described in epidermal melanocytes by Tarnowski (11). (b = basal lamina.) High magnification of area shown in Fig. 5b, IV shows detail of granules (g) having lamellae and lamellar scrolls characteristic of mast cell granules. (mi = mitochondrion.) Fig. 5a, I: × 14,500; II: × 38,000; III: × 120,000; Fig. 5b, I: × 10,000; II: × 40,000; III: × 24,000; IV: × 45,000.

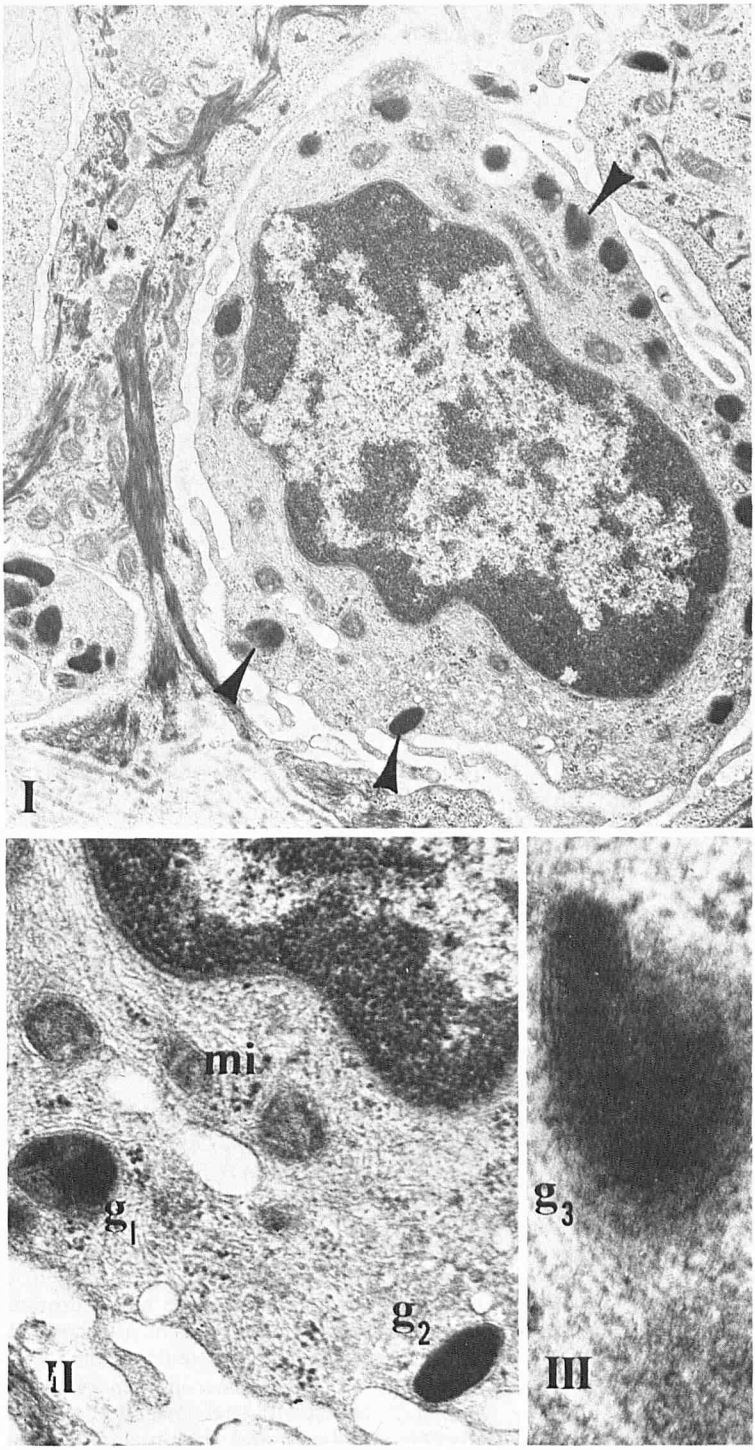

FIG. 5a.

anin. This work supports Bloch's concept of a specific dopa oxidase (25). Past studies indicating a mammalian tyrosinase in crude or partially purified preparations must be viewed with reservation, since none of these studies included controls for the presence of peroxidase and some did not include controls for metal catalysis. Burnett has described a purified "tyrosinase" preparation derived from mouse melanoma, but she presented data only for dopa oxidase activity (26). Wilgram

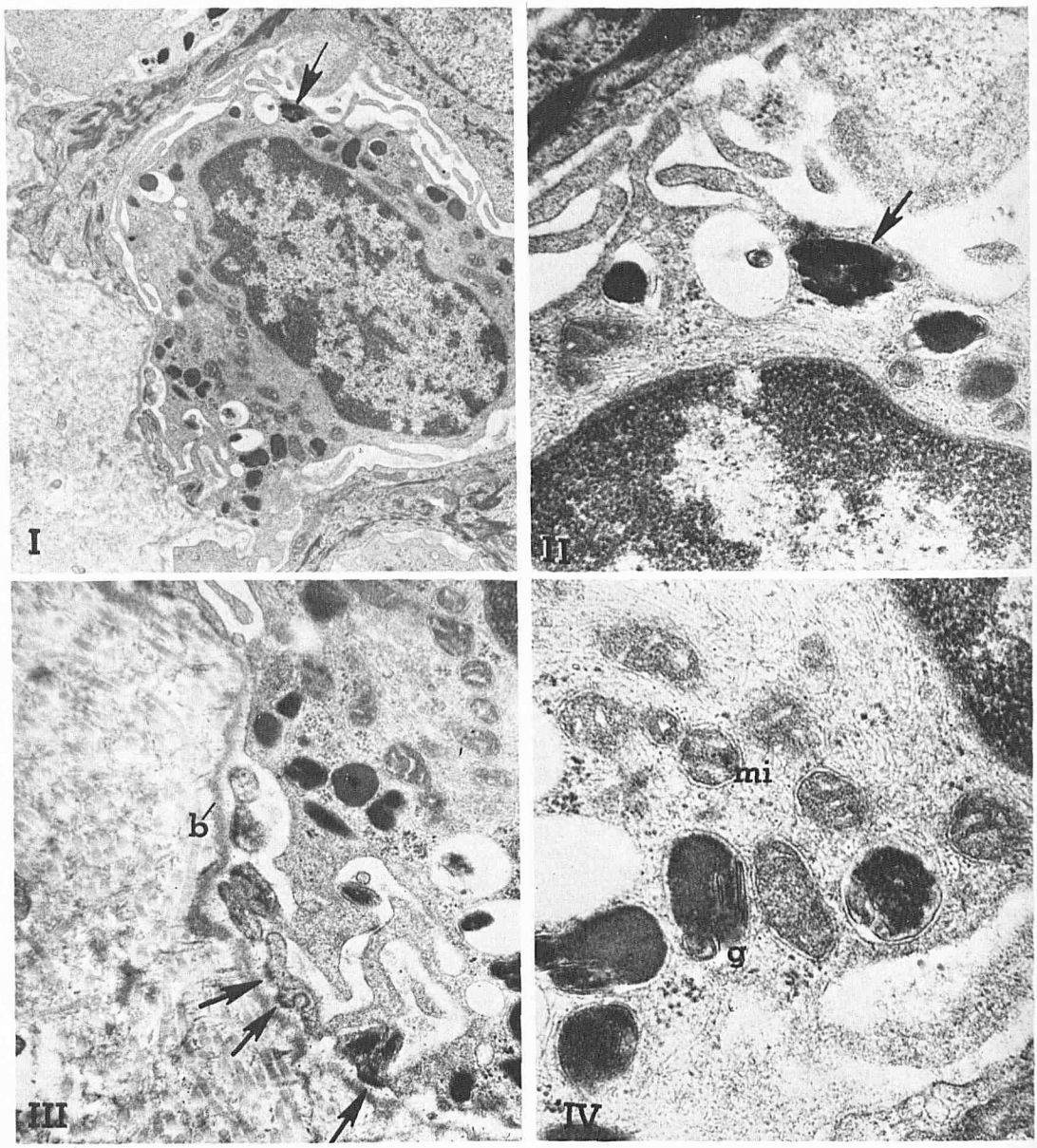

FIG. 5b.

has been unable to obtain a purified aerobic oxidase from mouse melanoma capable of oxidizing tyrosine to melanin (even with dopa co-factor), although he has been able to purify an aerobic dopa oxidase (27).

The presence of the melanogenic enzyme peroxidase is therefore a biochemical common denominator of mast cells and melanocytes.

We have proposed (16) that the peroxidase and the aerobic dopa oxidase may act synergistically in melanogenesis in melanocytes, even though peroxidase, acting alone, may mediate the synthesis of melanin, neuromelanin and lipofuscin *in vivo*. It remains to be determined whether intermediate cells such as those described in this study have both enzymes.

Observations of Sato et al. (*4*, *5*). Sato *et al.* concluded that mast cells could not synthesize melanin and that melanin present in mast cells represented phagocytized material. Their conclusions were based entirely on morphologic data; no histochemical or biochemical data were presented in their studies. In the 1969 study by Sato *et al.* it was asserted that melanin was observed in mast cells only as melanosome complexes within lysosome-like granules, although their published mi-

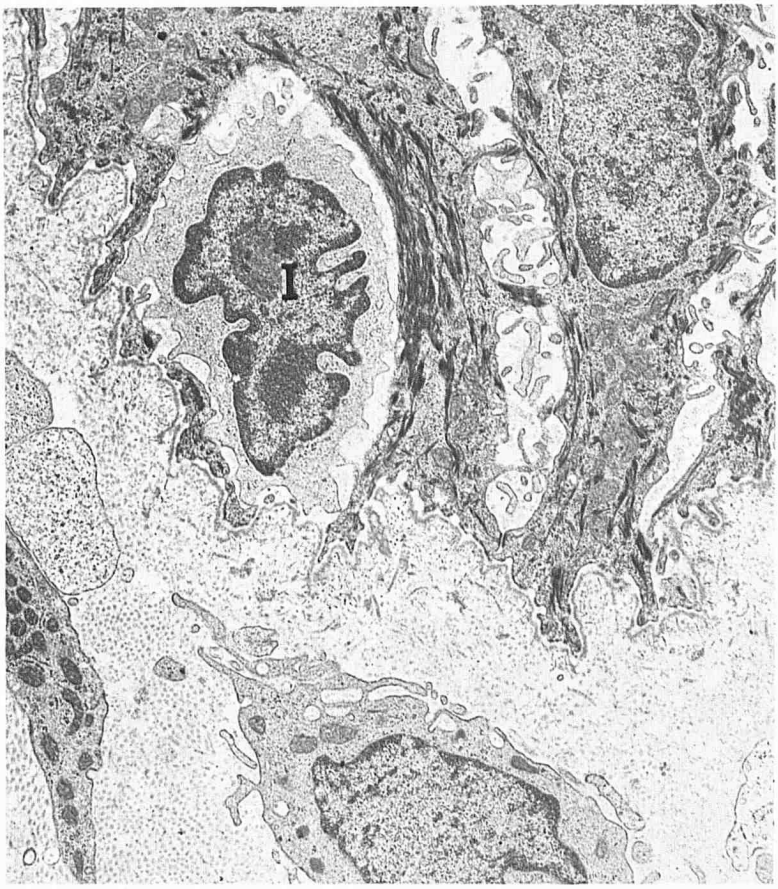

FIG. 6. Field shows indeterminate dendritic cell (I) in basal layer. The cell does not contain specialized cytoplasmic organelles. × 6400.

crographs also showed individual melanosomes in the cytoplasm of mast cells and groups of melanosomes which were not membrane-bound. In their 1971 study they identified melanosomes closely linked to mast cell granules, but ignored uniformly melanized mast cell granules present in their micrographs. As a likely explanation for the association of mast cell granules and melanosomes they proposed that the mast cell granule membrane and the unit cell membrane are continuous and that foreign material has an affinity for this common membrane.

The conclusions of Sato *et al.* do not seem to be justified. Although mast cells have phagocytic potential (28, 29) this does not prove that melanosome complexes in mast cells reflect the intake of exogenous melanin, and does not exclude either the presence of an endogenous melanogenic oxidase in mast cells or a histogenetic relationship to melanocytes.

Melanosome complexes and "melanophages." The origin of melanosome complexes is uncertain. Novikoff (30) has proposed that they may develop as a result of sequestration and autophagy of melanin produced by cells in which these complexes appear. Identification of acid phosphatase activity in melanosomes (30, 31) has led to the concept that melanosomes are lysosomes (30, 32) and that melanosomes may undergo self-degradation (32). The latter proposal raised the possibility that melanosome complexes represent a structural transformation of melanosomes associated with this process (Fig. 7a, insets). Larger melanosome complexes may result from the fusion of smaller complexes. Our study (3) in which dopa oxidase activity was noted in some "melanophages" by light microscopic histochemistry and the study of Novikoff *et al.* (30) in which dopa oxidase activity was noted in the Golgi-associated cisternae of smooth-surfaced endoplasmic reticulum (GERL) of "melanophages" by high resolution histochemistry, provide enzymatic evidence for the proposal that at least some "melanophages" may be melanocytes which have accumulated their own reaction product. This concept is supported by the range of dermal melanin-containing cells observed in our electron micrographs (Fig. 7). Some showed only melanosome com-

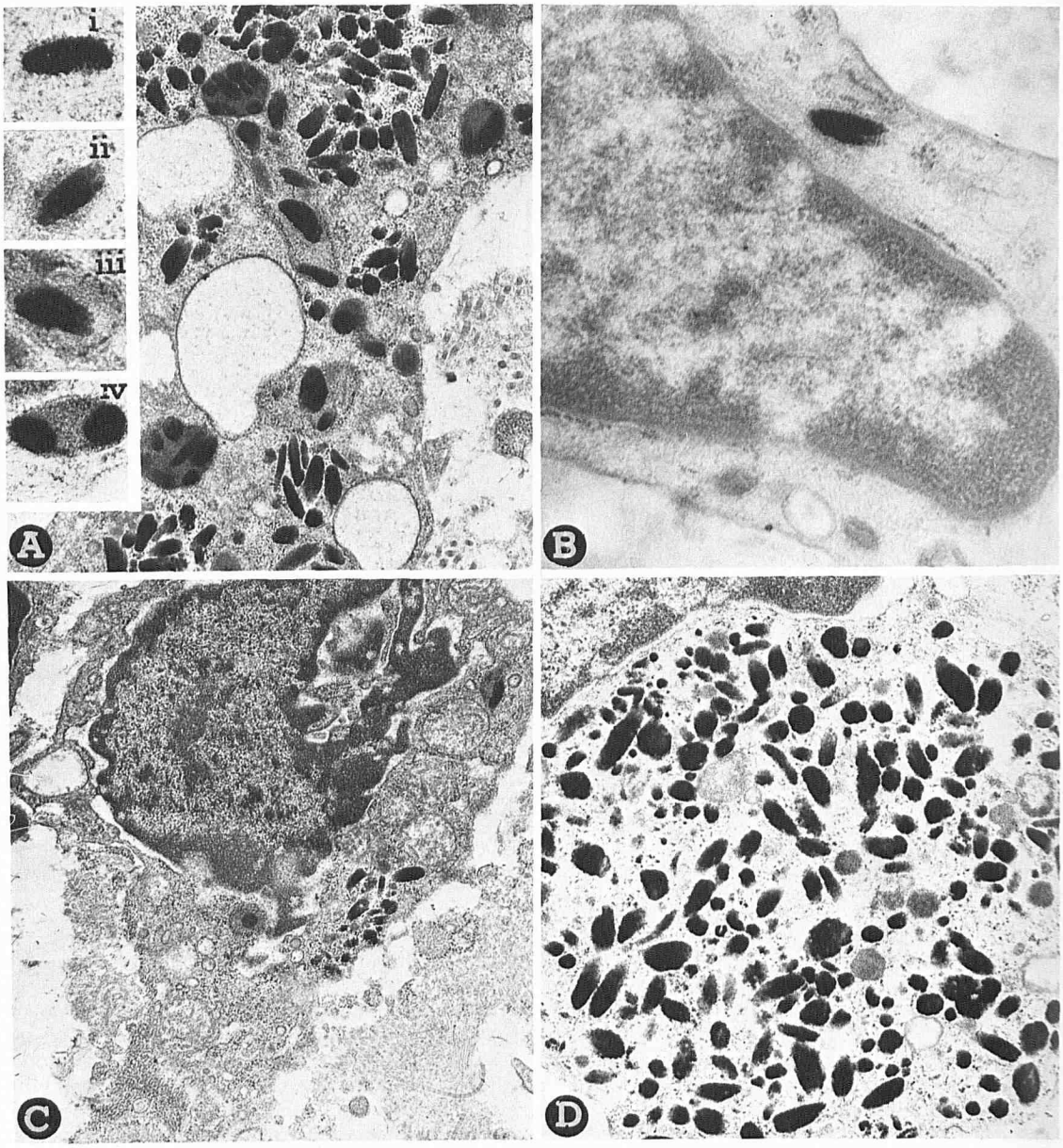

FIG. 7. Areas of 4 dermal cells are illustrated. Fig. 7a shows a cell with both individual melanosomes in various stages of melanization and melanosome complexes; cell in Fig. 7b shows a single partially melanized melanosome in its cytoplasm; Fig. 7c shows a cell with a group of individual melanosomes in its cytoplasm; Fig. 7d shows a cell with large numbers of individual melanosomes in various stages of melanization in its cytoplasm. Insets in Fig. 7a present selected organelles from a dermal cell: (i) shows an individual melanosome; (ii) shows an individual melanosome with a peripheral zone of finely granular material, but no outer membrane; (iii) shows a single melanosome in a membrane-bound structure containing finely granular material; (iv) shows a structure similar to that illustrated in (iii), but with two small foci of melanin. The sequence illustrates possible metamorphosis and self-degradation of melanosomes. Fig. 7a: × 23,000; insets: × 52,000; Fig. 7b: × 57,000; Fig. 7c: × 14,500; Fig. 7d: × 32,000.

plexes; some showed both melanosome complexes and individual melanosomes in various stages of melanization (the individual melanosomes ranged in maximum diameter from 0.2 microns to 0.5 microns); others showed only individual melanosomes. This concept is also supported by our observation and the observation of others (33) of melanosome complexes in epidermal melanocytes. "Melanophages" not showing dopa oxidase activity may represent late stages in which melanin synthesis has ceased and melanin degradation is dominant.

There are facts which are difficult to reconcile with the theory that "melanophages" are invari-

ably cells which phagocytize melanin which has been synthesized in other cells and released into the intercellular spaces ("pigmentary incontinence"). The rarity of melanosomes free in the dermis has been noted in our studies and in studies of others (34), even in areas where "melanophages" are present in abundance. In addition, the earlier stages (35, 36), which would be anticipated in the phagocytosis of melanin, have not been observed; melanosomes or fragments of melanocytes have not been seen in endocytotic vesicles or in phagosomes (true vacuoles, in contrast to melanosome complexes which are secondary lysosomes) in "melanophages." These early stages of heterophagocytosis of melanin have also been observed in mast cells.)

Although it is recognized that dermal melanocytes are normally present in mammals (37), including higher anthropoids (38), in man, pigmented cells in the dermis are assumed to be phagocytes (except in melanocytic neoplasms and dermal melanocytosis) despite the absence of convincing evidence and despite the divergence from the pattern present in other mammals.

It is of interest that acid phosphatase activity has also been observed in mast cell granules by histochemical (39) and biochemical (40) methods, and therefore the mast cell granule has also been considered to be a special form of lysosome.

Embryologic considerations.' The concept of a physiologic migration pattern of connective tissue cells into stratified squamous epithelium throughout life is supported by establishing the presence of a dendritic mast cell system in nonkeratinizing stratified squamous epithelium in various mammals (41) and Langerhans cells in keratinizing stratified squamous epithelium (42). Currently Langerhans cells are considered to be connective tissue cells, although their embryologic origin is uncertain. Until recently it was widely believed that there was a histogenetic relationship between Langerhans cells and melanocytes. Studies identifying Langerhans granules in normal histiocytes and histiocytes of histiocytosis X (43–45), as well as the embryologic study of Breathnach *et al.* (46) have led to the concept that Langerhans cells are epidermal macrophages of mesodermal origin, histogenetically distinct from melanocytes. In the study of Breathnach *et al.* Langerhans cells were identified in skin from embryos in which neural crest was removed. On the other hand, Reams *et al.* (47) were unable to demonstrate Langerhans cells in skin deprived of neural crest. These conflicting data may relate to the difficulty in identifying precise demarcation of embryologic areas and precise migration times (48). Zelickson (43) has presented ultrastructural evidence of intergrades between Langerhans cells and melanocytes and Zelickson and Mottaz (49) have presented evidence of a reciprocal numerical relationship between these cells.

Indeterminate dendritic cells are a normal component of epidermis (49–51) and may represent the pluripotential connective tissue cells which migrate into epithelium and differentiate into melanocytes, Langerhans cells or mast cells. Under certain conditions in keratinizing epithelium there may be a shift in the normal direction of differentiation resulting in the appearance of mast cells and intermediate forms described in this report.

The hypothesis that melanocytes have a connective tissue stem cell does not conflict with the evidence that melanocytes are of neural crest origin. There is embryologic evidence that neural crest contributes to mesenchyme (52, 53), including mesenchyme participating in the formation of the dermis (54, 55). Foulks has presented evidence that melanocyte precursors migrate from connective tissue into epithelial structures during adult life (56).

The precursors of the dendritic cell system in the epidermis may be in the loose connective tissue just beneath it. Falabella (57) has shown that repigmentation of depigmented areas can be produced by epidermal grafts obtained from suction blisters. In one of his cases repigmentation occurred even though the graft sloughed after 5 days. A possible explanation for this is that repigmentation was produced by the reestablishment of the subepidermal bed of competent melanoblasts by the proliferation of indeterminate cells.

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